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INSECTICIDES AND ALGAE

TOXICITY AND DEGRADATION

THE ONTARIO WATER RESOURCES COMMISSION

INSECTICIDES AND ALGAE: TOXICITY AND DEGRADATION

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SUMMARY

Relationships between three insecticides DDT, sevin and malathion and algae with respect to toxicity and degradation were investigated using materials obtained from a nearby waste stabilization pond and axenic cultures of *Chlorella pyrenoidosa* at pH 6.0 and 9.0.

DDT exhibited no toxic properties to either systems at concentrations up to 100 mg/l and was broken down only to a limited extent by algae. Sevin was found to be toxic to algae at concentrations of 0.1 mg/l and also received little degradation. Malathion exhibited no persistent inhibitory effect to algae and appeared to be metabolized fairly readily in comparison to the other two insecticides studied.

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INTRODUCTION

Since the release of the insecticide, DDT, in the mid 1940's, more than 200 basic organic chemicals have received widespread application to control various agricultural pests (4). Increasing concern about the pollutional effects of these materials is indicated by the number of symposia and scientific articles, which have appeared in recent years dealing with this subject (19, 20, 21, 29).

Although considerable information is available describing the effects of certain insecticides to various higher forms of aquatic life (19, 23, 29), only limited data exists concerning the impact of such chemicals on the first step of the aquatic food chain - the algae (18, 28) or the treatability of wastes containing these chemicals by an algal dependent system - the waste stabilization pond (8).

The following study was carried out to investigate relationships between three broadspectra insecticides and algae to determine what effect the entry of such materials may be expected to have on algal populations associated with waste stabilization ponds and the possible degradation of these chemicals in an algal environment. The three insecticides chosen were DDT - a chlorinated hydrocarbon, sevin - a carbamate, and malathion - an organophosphorus compound.

PROCEDURES

Axenic cultures of Chlorella pyrenoidosa Chick (#395 Indiana Culture Collection) (25) were grown in the following aerated media: KNO_3 - 500 mg/l, $MgSO_4$ - 500 mg/l, KH_2PO_4 - 140 mg/l, $FeSO_4$ - 20 mg/l, sodium citrate - 40 mg/l, at pH 9.0 (0.2 M Tris plus 0.1 M HCl) or pH 6.0 (0.2 M sodium maleate, 0.2 M NaOH) for seven days at 25°C in continuous illumination of 400 footcandles, before being used experimentally.

Toxicity experiments were carried out by adding the pesticide in 0.1 ml solvent (DDT - acetone, sevin and malathion -95% ethanol) to 50 ml aliquots of an algal culture contained in sterile cotton-plugged 125 ml flasks mounted on a shaker. The data, expressed as cells/ml, were obtained by averaging the number of cells in 10 fields using a haemacytometer.

Degradation experiments were carried out by adding 1 uc of radioactive labelled pesticide dissolved in the appropriate solvent to 500 ml values of a 7 day algal culture (approximately 6-7 x 10^6 cells/ml) having a media pH of 6.0. After the desired incubation period, the algae were harvested by centrifuging the culture for 60 minutes at 1,000 G. Recovery and chromatographic procedures for each insecticide were as follows:

DDT: DDT-phenyl ring ^{14}C (19.7 uc/mg-Radiochemical Centre, Amersham) was applied in 0.1 ml acetone, either as a coating on the walls of a sterile litre flask prior to the addition of the algae or directly to the algal aliquot. After harvesting, the algal pellet was transferred with warm 95% ethanol, dried, and extracted with three 50 ml volumes of

n-hexane, the combined hexane extracts receiving a sulphuric acid wash. Media were extracted with three 100 ml volumes of n-hexane, combined, washed with sulphuric acid and evaporated to dryness. Paper chromatographic analyses of extracts were carried out using Whatman #1 in the presence of Kelthane, DDD (Rohm and Haas) and DDE (Pesticide Repository, Perrine, Fla.) using a trimethylenepentane ascending method (16). Chromatograms were air dried and sprayed with a $AgNO_3$ chromogenic lamp.

SEVIN: 1-naphthyl-N-methyl (carbamate-^{14}C) (131 uc/mg-Nuclear Chicago) was added in 0.1 ml 95% ethanol to the algal culture. After harvesting, the algal pellet was rinsed twice with 10 ml aliquots of sterile media and transferred in 95% methanol and evaporated to dryness. The cell free media plus the above rinses were extracted with 200 ml, 100 ml and 100 ml volumes of chloroform: ethanol (v/v) (31), the combined extracts evaporated to dryness and the residue dissolved in 50% methanol, which was subsequently extracted with two 200 ml volumes of redistilled benzene. The benzene was dried over sodium sulfate and evaporated. Algal and media residues were dissolved in 50% methanol for chromatography.

Paper chromatographic examination was carried out with Whatman #2 using a methanol/water ascending technique (31) which was modified from a one to two directional system to effect better separation. Co-chromatography with labelled sevin and naphthol was carried out during the second run. After treatment with 1.5 N methanolic sodium hydroxide n-butanol, the chromatograms were examined under ultraviolet light.

MALATHION: Malathion-^{14}C (0,0 dimethyl - S (1,2 di ehtoxycarbonyl) ethyl (-1,-1 $2^{14}C$) phosphorodithioate) (6.64 uc/mg - Radiochemical Centre, Amersham) was added in 0.1 ml 95% ethanol to the algal culture. After harvesting, the algal pellet was rinsed twice with 10 ml sterile media, the algal suspension and media plus rinses extracted separately with 200 ml, 100 ml, 100 ml volumes of carbon tetrachloride (27) and the extracts evaporated to dryness. Ascending paper chromatography with Whatman #1 was carried out using a nonaqueous system with an immobile solvent of dimethylformamide: ethyl ether, 1:5 (v/v) and a 2,2,4 trimethylpentane mobile solvent (17). Extracts were dissolved in ethyl acetate (27) and co-chromatographed with malathion and malaoxon, the latter synthesized by bromination (7). After being air dried overnight, the chromatograms were sprayed with an alcoholic ammonia silver nitrate chromogenic agent and developed under germicidal lamps.

All chromatographic papers were prewashed in distilled water. Chromatograms, following development, were divided on the basis of Rf zones and assayed for radioactivity in a Tricarb scintillation counter.

All experiments were repeated at least twice.

RESULTS

Toxicity

Insecticide toxicity to algae was first tested by treating 50 ml aliquots of materials obtained from a nearby waste stabilization pond (pH 8.1) with 100 mg/l DDT, sevin, or malathion and examining the cultures after four days.

The results (Table I) indicate less than a 3 percent difference between DDT treated and untreated cultures with respect to the total number of algae, whereas sevin and malathion treatments reduced the algal population to less than 45 percent of their control.

Relationships between pesticides and algae were further investigated by noting the response of Chlorella pyrenoidosa when cultured in a media at pH 6.0 or 9.0 containing 100 mg/l of insecticide.

Over a seven day period (Table II), differences between DDT treated and untreated cultures did not exceed 10 percent at either pH. Treatment with sevin reduced the population up to 30 percent of the control, a greater toxicity occurring in the acid media. Malathion had no inhibitory effect in the acidic media and a reduction in the algal population evident by the fourth day in the alkaline media had disappeared by the seventh.

Responses of Chlorella to pesticide concentrations of less than 100 mg/l were also carried out. After four days, neither DDT nor malathion at these concentrations exhibited differences greater than 10 percent of their respective controls (Table III). Sevin appears to be inhibitory at concentrations of 0.10 mg/l and greater.

TABLE I

Effects of 100 mg/1 DDT, sevin or malathion on algae in samples from a waste stabilization pond after four days-cells/ml x 10^4.

Algae	Acetone	DDT	Ethanol	Sevin	Malathion
Scenedesmus	18	8	15	0	5
Chlorella	792	789	500	152	469
Ankistrodesmus	806	867	634	295	0
Euglena	9	3	10	0	24
Total	1625	1667	1159	447	498

TABLE II

Effects of 100 mg/l of DDT, sevin and malathion on the growth of Chlorella A) media pH 6.0 B) media pH 9.0 - cells/ml x 10^6.

A) Time in Days	Solv.	DDT	Solv.	Sevin	Solv.	Mal.
1	8.56	7.99	8.86	6.91	8.84	7.75
2	9.42	9.57	9.61	7.57	9.59	8.77
3	9.58	9.45	10.12	7.25	9.46	9.07
5	10.37	9.57	9.71	6.33	9.42	8.76
7	9.31	9.36	9.56	6.66	9.43	8.83

B) Time in Days	Solv.	DDT	Solv.	Sevin	Solv.	Mal.
1	6.91	6.58	9.27	8.92	9.27	7.57
2	7.30	7.18	9.87	8.42	9.87	6.88
3	7.37	7.15	9.62	8.36	9.62	6.35
5	7.37	7.15	9.62	8.07	9.61	7.86
7	7.64	7.16	9.83	8.25	9.83	9.22

TABLE III

Effects of DDT, sevin or malathion on the growth of Chlorella after four days, media - pH 6.0 - cells/ml x 10^6.

Pesticide mg/l	DDT	Sevin	Malathion
0	10.74	9.36	9.36
10^{-2}	10.91	9.56	8.79
10^{-1}	10.66	8.60	8.43
10^{0}	9.93	7.81	8.84
10^{1}	10.35	7.87	8.54

In view of the responses of Chlorella to malathion (Table II) the effects of 100 mg/l malathion on pond materials after seven days exposure were also examined (Table IV). No inhibitory effect on the basis of total cell count is evident.

Degradation

The action of Chlorella on DDT was investigated by applying 1 uc DDT-^{14}C in acetone (about .05 mg) to the walls of sterilized flasks and allowing the solvent to evaporate before adding a 500 ml aliquot of algae, media pH 6.0. After one, four and seven days the cultures were harvested. The average efficiency of recovery of radioactivity was about 5 percent.

As a control measure, an equivalent volume of algae killed by autoclaving was treated in a similar manner and extracted after four days.

Comparing the results of experiments with living algae (Table V) to those with dead algae (Table VI) shows that degradation of DDT-^{14}C only occurred in the presence of live algae.

As the efficiency of recovery of radioactivity in the above experiments was quite low, degradation of the insecticide was examined by applying DDT-^{14}C dissolved in acetone, directly to 500 ml aliquots of cultures. After seven days, the cultures were harvested. The efficiency of recovery was 65 percent.

The results of these experiments, Table VII, do not illustrate any significant conversion of DDT-^{14}C.

TABLE IV

Effects of Malathion, 100 mg/l, on algae from a waste stabilization pond, after seven days - No. of cells/ml x 10^4.

Algae	Solvent	Malathion
Scenedesmus	6	1
Chlorella	0	353
Euglena	708	492
Schroderia	46	22
Total	760	868

TABLE V

Percentage distribution of recovered radioactivity after incubation of *Chlorella* with DDT-^{14}C, applied via the vessel walls.

Time of Exposure Days	Rf Kelthane^{R}	Rf DDD	Rf DDT	Rf DDE
1	5.1	7.7	78.7	3.6
4	7.0	22.7	58.1	1.3
7	5.8	35.7	36.7	2.9

TABLE VI

Percentage distribution of recovered radioactivity after incubation of dead Chlorella with DDT-^{14}C, applied via the vessel walls.

Time of Exposure Days	Rf Kelthane^{R}	Rf DDD	Rf DDT	Rf DDE
4	4.7	4.8	88.2	0.8

TABLE VII

Percentage distribution of recovered radioactivity after incubation of Chlorella with DDT-^{14}C, applied in acetone.

Time of Exposure Days	Rf Kelthane^{R}	Rf DDD	Rf DDT	Rf DDE
7	3.0	5.0	83.6	5.5

The degradation of sevin by Chlorella was investigated by applying 1 uc 1-naphthyl-N methyl carbamate-^{14}C (about 7.6 ug) in 0.1 ml 95 percent ethanol to a 500 ml volume of Chlorella, media pH 6.0. After one, four and seven days, the treated cultures were harvested. The average efficiency of recovery of radioactivity was about 50 percent.

The results, Table VIII, show that after seven days, about 87 percent of the radioactivity recovered was in the form of sevin. Amounts of radioactivity associated with algae increased with time, the largest increment occurring in that portion present in a form other than sevin.

Malathion breakdown by Chlorella was investigated by applying 1 uc malathion-^{14}C (about 0.15 mg) in 0.1 ml 95 percent ethanol to a 500 ml volume of algal culture, media pH 6.0. After one, four and seven days, the cultures were harvested. The average efficiency of radioactivity recovery was about 24.6 percent after one day, 20.4 after four days and 9.7 percent after seven days.

The results, Table IX, indicate that after seven days, 67 percent of the recovered radioactivity was in the form of malathion. Amounts of radioactivity associated with the algae increased with time, although the percentage of malathion and a possible metabolite malaoxon did not vary appreciably.

TABLE VIII

Percentage distribution of radioactivity recovered after incubation of Chlorella with Sevin-^{14}C.

Incubation Period Days	Rf Recovered as Sevin	Recovered in Algae	Rf as Sevin in Algae	Non-migratory in Algae
1	92.8	4.8	41.4	35.0
4	90.2	7.0	18.1	63.0
7	87.5	9.6	13.1	76.6

TABLE IX

Percentage distribution of recovered radioactivity after incubation of Chlorella with Malathion-^{14}C, media - pH 6.0.

Time Days	Rf Malathion in Media	Recovered in Algae	Non-migratory in Algae	Rf Malathion	Rf Malaoxon
1	95.7	10.4	6.6	67.3	3.4
4	93.2	11.6	7.4	62.7	6.6
7	76.0	23.7	13.8	59.9	7.1

DISCUSSION

The results of the above experiments show that DDT concentrations up to 100 mg/l have no effects on the growth of Chlorella (Table I, II, III) and that degradation of the insecticide only occurs in the presence of living algae (Table V, VII).

The apparent lack of agreement regarding the fate of DDT-^{14}C with living algae (Table V, VII) is probably due to the low solubility of the pesticide in water - 1.2 ug/l (3). Treatment of algal cultures with quantities of insecticide in excess of this amount (Table VII) will result in the formation of a DDT-^{14}C precipitate which, when recovered during harvesting of the cultures, would tend to mask any metabolic conversion of the pesticide, which may have occurred.

The data in Table V, on the other hand, are based only on the recovery of radioactive materials which originated from aqueous soluble DDT-^{14}C, and show that Chlorella is capable of metabolizing the insecticide, possibly by dehydrochlorination to DDD or a similar material, as has been determined with various bacteria (2, 9, 10, 15, 26, 30), yeast (11) and actinomycetes (5).

The lack of influence of DDT on the growth of Chlorella pyrenoidosa, also observed by Palmer and Maloney (18) and Ukeles (28), would appear to result from the low solubility of the compound in water and the ability of the algae to degrade the pesticide.

Treatment of axenic cultures of Chlorella with sevin, 100 mg/l, did not demonstrate the degree of growth inhibition noted in experiments with materials from a waste stabilization pond (Table I). These differences may reflect a higher degree of sensitivity of the pond algae to the pesticide as well as the involvement of numerous other factors, such as bacteria, which were not present in the axenic culture. Results of these experiments (Table III) are in agreement with observations of Ukeles (28) who also found that concentrations in excess of 0.1 mg/l were toxic to Chlorella spp.

As sevin is hydrolized under alkaline conditions (24), the relationship between pesticide toxicity and pH (Table II) is more likely due to the loss in the persistence of sevin at the higher pH, an effect previously noted by Lichenstein et al (13), rather than an increase in the sensitivity of the test organism at the low pH.

Sevin has an aqueous solubility of 40 mg/l (12), therefore the quantities applied in degradation experiments should have remained in a soluble form. The high recovery of sevin-^{14}C, about 87 percent after seven days, should not be due therefore to a masking of metabolic conversions by the recovery of insoluble precipitates as with DDT-^{14}C. Although the results would suggest littlé conversion of applied sevin, examination of the algal extracts does indicate an increasing uptake of radioactivity with time with a concomitant increase in quantities of radioactive material which did not migrate with the chromatographic system used in this study.

Identification of 1-napthol as a by-product of sevin metabolism (31) was not possible as the radioactive carbon atom was not incorporated into the naphthol portion of 1-naphthyl-N methyl carbamate nor was there any colour response to the chromogenic agent. Identification of non-migratory materials was not attempted.

Malathion applications up to 100 mg/l appear to have no effect on the growth of algae in an acid media (Table II, III) nor a lasting inhibitory influence on the total standing crop of algae in an alkaline environment (Table II, IV). Malathion in this regard appears to be similar to another organophosphorus insecticide, Thimet, which at much higher concentrations also had no influence on the growth of Chlorella after an eight day exposure (1). These results are also in agreement with observations of Gloyna and Thirmulthi (8) that the entry of a slug of this pesticide into materials from a waste stabilization pond will result in a qualitative alteration in the algal community.

Malathion has an aqueous solubility of 145 mg/l (27) therefore all malathion-^{14}C applied in degradation experiments would be expected to remain in solution.

Malathion conversion may occur by hydrolyses, which under alkaline conditions yields 0,0 dimethyldithiophosphate, fumerate and ethanol, or be enzymatically altered by the action of carboxyesterases. Metabolic conversions in lettuce resulted in the formation of malaoxon and three unknowns (6), in wheat to malaoxon (22) and to various carboxylic acids in the fungus Trichoderma and yeast (14). Chlorella would also appear to be capable of a limited conversion of malathion to malaoxon (Table XI).

In degradation studies with sevin, the efficiency of recovery of radioactivity remained fairly uniform with time, whereas with malathion-^{14}C, this efficiency decreased quite markedly with time. Extrapolation to zero time suggests a maximum possible recovery of only 25 percent or 37 ug of the initially applied 150 ug. A recovery of 11.03 ug after seven days would imply a degradation of about 70 percent of the original material of which about 37 percent had disappeared, probably in the form of carbon dioxide as a product of metabolism. This interpretation would suggest that the degradation of malathion by Chlorella is in a lower order of magnitude than that of either the fungus Trichoderma or yeast where up to 70 percent of applied malathion was converted within 25 hours (14) and of lettuce where 97 percent was metabolized in 48 hours (6).

CONCLUSIONS

The results of the above investigations indicate that the three pesticides vary in their degree of toxicity to algae and also in the extent to which they are degraded in the presence of algae.

DDT exhibited no toxic properties up to a concentration of 100 mg/l and received only slight degradation. Sevin is toxic at concentrations above 0.1 mg/l and is not altered appreciably in an acidic media. Malathion appears to receive extensive conversion and although capable of altering the composition of a mixed algal community, did not display a persistent inhibitory effect.

Extending the results of these experiments to the typically alkaline waste stabilization pond and its algal community it is suggested that the entry of DDT, up to 100 mg/l, is not likely to be toxic nor will the material be degraded by algae. A similar quantity of sevin would seriously reduce the efficiency of the pond with conversion more likely to occur by alkaline hydrolysis rather than algal metabolic processes. An equivalent slug of malathion, although temporarily interrupting, the operation of the pond, could be expected to be broken down rapidly by chemical and metabolic reactions.

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REFERENCES

1. Amhed, M. K., Casida, J. E., Metabolism of some organophosphorus insecticides by microorganisms. J. Econ. Entomol. 51: 59 (1958).

2. Barder, P. S., Morrison, F. O., Whitaker, R. S., Conversion of DDT to DDD by Proteus vulgaris, a bacterium isolated from the intestinal flora of a mouse, Nature 205: 621 (1965).

3. Bowman, M. C., Acree, F., Jr., Corbett, M. K., Solubility of Carbon - 14 DDT in Water, Jour. Agric. Food Chem. 8: 406 (1960).

4. Carson, Rachel, Silent Spring, The Riverside Press, Boston (1962).

5. Chacko, C. I., Lockwood, J. L., Zobik, M., Chlorinated hydrocarbon pesticides-degradation of microbes, Science 154: 893 (1966).

6. Coffin, D. E., Oxidative metabolism and persistence of parathion and malathion on field-sprayed lettuce. Jour. Assoc. Off. Agric. Chem. 49: 1018 (1966).

7. Coffin, D. E., McKinley, W. P., Determination of parathion, methyl parathion, EPN and their oxons in some fruit and vegetable crops. Jour. Assoc. Off. Agric. Chem. 46: 223 (1963).

8. Gloyna, E. F., Thirumurthi, D., Suppression of photosynthetic oxygenation, Water and Sewage Works Jour. 114: 83 (1967).

9. Hill, D. W., McCarty, P. L., Anaerobic degradation of selected chlorinated hydrocarbon pesticides, Jour. Water Poll. Cont. Fed. 39:1259 (1967).

10. Johnson, B. T., Goodman, R. N., Goldberg, H. S., Conversion of DDT to DDD by pathogenic and saprophytic bacteria associated with plants, Science, 157: 560 (1963).

11. Kallman, B. J. and Andrews, A. K., Reductive dechlorination of DDT to DDD by yeast. Science, 141: 1050 (1963).

12. Knack, J. B., Tallant, M. J., Bartley, W. J., Sullivan, L. J., The metabolism of Carbaryl in the rat, guinea pig and man, Jour. Agric. Food, Chem. 13: 537 (1965).

13. Lichtenstein, E. P., Schulz, K. R., Skrentny, R. F., Tsukano, Y., Toxicity and fate of insecticide residues in water. Arch. Environ. Health 12: 199 (1966).

14. Matsumura, E., Bousch, G. M., Malathion degradation by Trichoderma viride and a Pseudomonas species, Science 153: 1278 (1966).

15. Mendell, J. L., Klein, A. K., Chen, J. T., Walton, M. S. Metabolism of DDT and some other chlorinated organic compounds by Aerobacter aerogenes. Jour. Assoc. Off. Agric. Chem. 50:897 (1967).

16. Mitchell, L. C., Separation and identification of chlorinated organic pesticides by paper chromatography, XI, Jour. Assoc. Off. Agric. Chem. 41: 781 (1958).

17. Mitchell, L. C., Separation and identification of eleven organophosphate pesticides by paper chromatography. Jour. Assoc. Off. Agric. Chem. 43: 810 (1960).

18. Palmer, C. M. and Maloney, T. E., Preliminary screening for algicides, Ohio Jour. Sci. 55: 1 (1955).

19. Pesticides in Soil and Water, U. S. Department of Health, Education and Welfare, 99-WP-17 (1964).

20. Organic Pesticides in the Environment, Symposium chairmen, A. A. Rosen and H. F. Kraybill, American Chemical Society, Washington (1966).

21. Research in Pesticides, Proceedings of the Conference on Research Needs and Approaches to the Use of Agricultural Chemicals, ed. C. O. Chichester (1965) Academic Press, New York.

22. Rowlands, D. G., In vitro and in vivo oxidation and hydrolysis of malathion by wheat grain esterases. Jour. Sci. Food Agric. 16: 325 (1965).

23. Spiller, D., A digest of available information on the insecticide malathion, Advances in Pest Control Research, 4: 249 (1961).

24. Stanbury, H. A., Miskus, R., Chapt. 39, Sevin, in Analytical Methods for Pesticides, Plant Growth Regulators and Food Additives, Vol. II ed. G. Zweig, Academic Press (1964).

25. Starr, R. C., The culture collection of algae at Indiana University, Am. Jour. Botan., 51: 1013 (1964).

26. Stenersen, J. H. V., DDT metabolism in resistant and susceptible stable flies and bacteria, Nature, 207: 660 (1965).

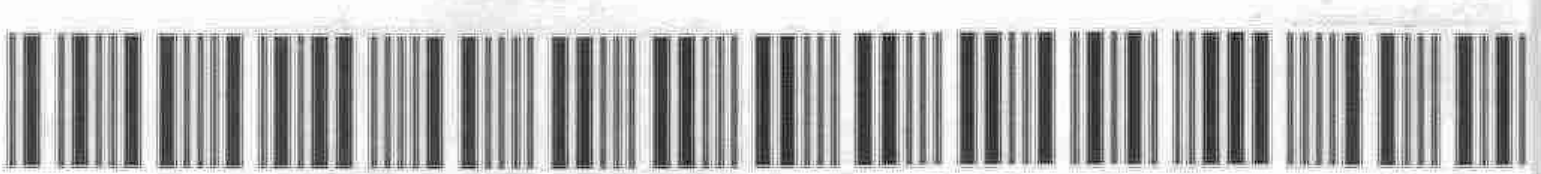
27. Sutherland, G. L., Analytical Methods for Pesticides, Plant Growth Regulators and Food Additives, Vol. II, Chapter 25, Malathion, Ed. G. Zweig, Academic Press, (1964).

28. Ukeles, R., Growth of pure cultures of marine phytoplankton in the presence of toxicants, Appl. Microbiol., 10:532 (1962).

29. Water Quality Criteria, Publ. No. 3-A, Chapter IX, Ed. J. E. McKee and H. W. Wolf (1963) State Water Quality Control Board, Sacramento, California.

30. Wedemeyer, G. A., Dechlorination of DDT by Aerobacter aerogenes. Science, 152: 647 (1966).

31. Zweig, G., Archer, T. E., Residue determinations of Sevin (1-Naphthyl - N - methylcarbamate) in wine by cholinesterase inhibition and paper chromatography. Jour. Agric. Food Chem., 6: 910 (1958).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